

Claims:

- 5 1. Method for producing an immunoglobuline having Fc receptor activity and/or complement activation activity which immunoglobuline molecule when secreted from a vertebrate host cell comprises at least a first and a second polypeptide chain which two polypeptide chains are different, comprising the steps of
- 10 a. expressing in a vertebrate host cell having Golgi-only or late-Golgi-only resident furin family endoprotease activity an fusion polypeptide comprising a secretion targetting sequence directing the polypeptide to the secretory pathway and further comprising at least the first and second polypeptide sequences and at least one cleavage site for the said endoprotease activity
- 15 b. having the fusion polypeptide cleaved in the cells by the furin family endoprotease activity into the first and second polypeptide chains and
- c. harvesting the secreted immunoglobuline.
- 20 2. Method according to claim 1, characterized in that the Ig molecule comprises at least a hinge domain, a CH2 and a CH3 domain.
3. Method according to claim 1, characterized in that the Ig molecule is a standard Ig
- 25 molecule comprising a VL, a VH, a CL, a CH1, CH2 and CH3 domain and a hinge domain.
4. Method according to claim 3, characterized in that the first polypeptide is an Ig-Light Chain (L) and in that the second polypeptide is an Ig-Heavy Chain (H).
- 30 5. Method according to claim 1 or 4, characterized in that the fusion polypeptide comprises the sequences of the first and second polypeptide separated by a linker.
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6. Method according to claim 4, characterized in that the Light and Heavy Chain are separated by a linker and that the linker is cleaved off from both Heavy and Light Chain by the furin family endoprotease activity.
- 5 7. Method according to claim 6, characterized in that the fusion polypeptide comprises at least two cleavage sites.
8. Method according to claim 1, characterized in that the furin family endoprotease activity is an activity naturally present in that host cell line.
- 10 9. Method according to claim 1, characterized in that the host cell is devoid of furin family endoprotease activity in the endoplasmic reticulum.
10. Method according to claim 9, characterized in that the host cell comprises at least one first recombinant furin family endoprotease activity and is devoid of activity from that first recombinant endoprotease in the endoplasmic reticulum.
- 15 11. Method according to claim 10, characterized in that the first recombinant endoprotease is furin endoprotease or lymphoma proprotein convertase or a functional variant thereof.
- 20 12. Method according to claim 10 or 11, characterized in the host cell is a CHO cell.
13. Method according to claim 1, characterized in that the furin family endoprotease activity is a constitutive endoprotease activity.
- 25 14. Method according to claim 1, characterized in that the host cell line is a mammalian cell line.
- 30 15. Method according to claim 14, characterized in that the at least one recombinant furin family endoprotease activity stems is a homologously expressed mammalian furin family endoprotease naturally present in that host cell line which further is an

constitutive furin family endoprotease or furin family endoprotease belonging to constitutive secretion, in this way achieving an elevated expression level of the natural gene product in its native host cell environment.

5 16. Method according to claim 15, characterized in that the mammalian host cell line are CHO cells.

10 17. Method according to claims 1 or 7, characterized in that the cleavage sites is an contiguous tetrapeptide sequence comprising at least three basic residues selected from the group consisting of arginine and lysine.

15 18. Method according to claim 17, characterized in that the tetrapeptid sequence comprises four basic residues selected from the group consisting of arginine and lysine.

20 19. Method according to claim 7, characterized in that the linker is a non-naturally occurring amino acid sequence.

25 20. Method according to claim 19, characterized in that the linker comprises at least 20 amino acids.

30 21. Method according to claim 20, characterized in the linker comprises one or several oligomers consisting of only glycine and either serine, threonine or both.

35 22. Method according to claim 21, characterized in that the linker consists of one or several oligomers consisting of only glycine and either serine, threonine or both.

40 23. Method according to claim 22, characterized in that the linker comprises at least >60% glycine residues.

45 24. Method for producing an immunoglobuline molecule having Fc receptor activity and/or complement activation activity which immunoglobuline molecule comprises multiple

copies each of at least a first and a second polypeptide chain when secreted from a host cell and which two polypeptide chains are different, comprising the steps of:

- 5 a. expressing in a vertebrate host cell having Golgi or late-Golgi-resident furin family endoprotease activity an fusion polypeptide comprising a secretion targetting sequence directing the polypeptide to the secretory pathway and further comprising at least the first and second polypeptide sequences and at least one cleavage site for the said endoprotease activity
- 10 b. having the fusion polypeptide cleaved in the cells by the furin family endoprotease activity into the first and second polypeptide chains and
- 15 c. harvesting the secreted, functional immunoglobuline molecule.

25. Method for producing an immunoglobuline having Fc receptor activity and/or complement activation activity which immunoglobuline molecule when secreted from a host cell comprises at least a first and a second polypeptide chain which two polypeptide chains are different, comprising the steps of

- 20 a. expressing in a yeast host cell having kex2 endoprotease activity and humanized N-glycosylation an fusion polypeptide comprising a secretion targetting sequence directing the polypeptide to the secretory pathway and further comprising at least the first and second polypeptide sequences and at least one
- 25 cleavage site for the said kex-2 endoprotease activity
- b. having the fusion polypeptide cleaved in the cells by the kex-2 endoprotease activity into the first and second polypeptide chains and
- 30 c. harvesting the secreted immunoglobuline molecule

26. Method according to claim 47, characterized in that the yeast host cell is *Saccharomyces cerevisiae* or a methylotropic yeast, preferably a *Pichia* species.

27. Method according to claim 48, characterized in that the yeast host cell is *Saccharomyces cerevisiae*.

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28. Method according to claim 47, characterized in that the meaning of humanized N-glycosylation is that the yeast host cell is devoid of outer chain glycosylation and preferably is capable at least of generating biantennary oligo-mannose N-glycan structures and/or complex N-glycan core structures comprising at least an biantennary Asn-GlcNAc₄ Man₃ moiety.

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29. Method according to claim 28, characterized in that the N-glycan comprises an bisecting N-acetyl-glucosamine residue enhancing Fc-receptor activity of the glycosylated antibody thus produced from the host cell.

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30. Host cell for producing an immunoglobuline having Fc receptor activity and/or complement activation activity which said immunoglobuline molecule when secreted from the host cell comprises at least a first and a second polypeptide chain which two polypeptide chains are different, said host cells expressing Golgi-only or late Golgi only resident furin family endoprotease activity and further expressing a fusion polypeptide comprising a secretion targeting sequence directing the polypeptide to the endoplasmic reticulum and hence the secretory pathway for secretion to the extracellular space, which fusion polypeptide comprises at least the first and second polypeptide sequences and at least one cleavage site for the said endoprotease activity.

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31. Host cell according to claim 30, characterized in that the immunoglobuline comprises multiple copies each of at the least the first and second polypeptide chain when secreted from the host cells.

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32. Host cell according to claim 31, characterized in that the host cell is a fungal host cell, preferably a yeast host cell, having kex2 endoprotease activity and humanized N-glycosylation and wherein the at least one cleavage site is cleavable by the said kex-2 endoprotease activity

33. Host cell according to claim 32, characterized in that the the kex-2 endoprotease activity is a natural kex-2 endoprotease activity present in that host cell.
- 5 34. . Host cell according to claim 33, characterized in that the natural kex-2 endoprotease activity is vector-borne in said host cell.
35. . Host cell according to claim 30 or 31, characterized in that the host cell is a vertebrate host cell.
- 10 36. . Host cell according to claim 36, characterized in that the host cells is a mammalian host cell.
37. Host cell according to any of the preceding claims, characterized in that the Ig molecule comprises at least a hinge domain, a CH2 and a CH3 domain.
- 15 38. . Host cell according to claim 38, characterized in that the Ig molecule is a standard Ig molecule comprising a VL, a VH, a CL, a CH1, CH2 and CH3 domain and a hinge domain.
- 20 39. Host cell according to claim 41, characterized in that the first polypeptide is an Ig-Light Chain (L) and in that the second polypeptide is an Ig-Heavy Chain (H).
40. Host cell according to any of the preceding claims, characterized in that the fusion polypeptide comprises the sequences of the first and second polypeptide separated by a linker.
- 25 41. Host cell according to claim 40, characterized in that the Light and Heavy Chain are separated by a linker by means of at least two cleavage sites and that the linker is cleavable off from both Heavy and Light Chain by the furin family endoprotease activity.
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42. Host cell according to claim 30, characterized in that the furin family endoprotease activity is an activity naturally present in that host cell line.
- 5 43. Host cell according to claim 36, characterized in that the host cell is devoid of furin family endoprotease activity in the endoplasmic reticulum.
- 10 44. Host cell according to claim 36, characterized in that the host cell comprises at least one first recombinant furin family endoprotease activity and is devoid of activity from that first recombinant endoprotease in the endoplasmic reticulum.
45. Host cell according to claim 45, characterized in that the first recombinant endoprotease is furin endoprotease or lymphoma proprotein convertase or a functional variant thereof.
- 15 46. Host cell according to claim 37 or 45, characterized in that the host cell is a CHO cell.
47. Host cell according to claim 36 or 37, characterized in that the furin family endoprotease activity is a constitutive endoprotease activity.
- 20 49. Host cell according to claim 45, characterized in that the at least one recombinant furin family endoprotease activity stems from a homologously expressed mammalian furin family endoprotease naturally present in that host cell line which further is an constitutive furin family endoprotease or furin family endoprotease belonging to constitutive secretion, in this way achieving an elevated expression level of the natural
- 25 gene product in its native host cell environment.
50. Host cell according to any of the preceding claims, characterized in that the cleavage sites is an contiguous tetrapeptide sequence comprising at least three basic residues selected from the group consisting of arginine and lysine.
- 30 51. Host cell according to claim 50, characterized in that the tetrapeptid sequence comprises four basic residues selected from the group consisting of arginine and lysine.

52. Host cell according to claim 50, characterized in that the linker is a non-naturally occurring amino acid sequence.

5 53. Host cell according to claim 50, characterized in that the linker comprises at least 20 amino acids.

54. Host cell according to claim 50, characterized in the linker comprises one or several oligomers consisting of only glycine and either serine, threonine or both.

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55. Host cell according to claim 54, characterized in that the linker consists of one or several oligomers consisting of only glycine and either serine, threonine or both.

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56 Host cell according to claim 55, characterized in that the linker comprises at least >60% glycine residues.

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57 Method for producing an immunoglobuline having Fc receptor activity and/or complement activation activity which immunoglobuline molecule when secreted from a vertebrate host cell comprises at least a first and a second polypeptide chain which two polypeptide chains are different, comprising the steps of

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a. expressing in a vertebrate host cell having Golgi-only or late-Golgi-only resident subtilisin/kexin family endoprotease activity an fusion polypeptide comprising a secretion targetting sequence directing the polypeptide to the secretory pathway and further comprising at least the first and second polypeptide sequences and at least one cleavage site for the said endoprotease activity

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b. having the fusion polypeptide cleaved in the cells by the subtilisin/kexin family endoprotease activity into the first and second polypeptide chains and

c. harvesting the secreted immunoglobuline.